Original Articles

Microsattellite Polymorphism and Parentage Control in Thai Domestic Elephants *(Elephas maximus)*

Duangsmorn Suwattana[1*] Weerapong Koykul[2] Jutharat Jirasupphachok[1] Sumolya Kanchanapangka[2]

Abstract

Polymorphism of microsatellite DNA in Thai domestic elephants (Elephas maximus) was studied using five genetic markers including LaT05, LaT07, LaT16, LaT17 and LaT26, in order to determine the efficiency of such markers in parentage identification. Eleven elephants from 5 families and 10 unrelated ones located in the northern, northeastern and central parts of Thailand were tested. It was found that LaT05, LaT16, LaT17 and LaT26 possessed marker sizes of 250-500 bp with numbers of alleles ranging from 4-13, heterozygosity 0.62-0.88 and PIC 0.56-0.87 whereas LaT07 could not be detected in all specimens used. LaT05 and LaT26 appeared to be the most desirable markers with PIC=0.87. Parentage identification using the 4 genetic markers showed the results corresponding to the family history and pedigree record of all specimens with up to 99.74% of efficiency and accuracy. It was concluded that LaT05, LaT16, LaT17 and LaT26 all together could be used in parentage identification.

Keywords : microsatellite, polymorphism, parentage, *Elephas maximus*

[1]Department of Animal Husbandry, [2]Department of Veterinary Anatomy, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand.

[*]Corresponding author

บทคัดย่อ

การตรวจหาความสัมพันธ์ทางสายเลือดในช้างไทยโดยใช้ความหลากหลายของไมโครแซทเทลไลท์

ดวงสมร สุวัฑฒน[1*] วีระพงศ์ โกยกุล[2] จุฑารัตน์ จิระศุภโชค[1] สุมลยา กาญจนะพังคะ[2]

ศึกษาความสัมพันธ์ทางสายเลือดของช้างไทยโดยใช้ความหลากหลายของไมโครแซทเทลไลท์ ดีเอ็นเอ จำนวน 5 ตัว คือ LaT05, LaT07, LaT16, LaT17 และ LaT26. เก็บตัวอย่างเลือดเพื่อสกัดดีเอ็นเอจากช้าง 5 ครอบครัว จำนวน 11 ตัว และจากช้าง 10 ตัว ที่ไม่มีความสัมพันธ์ทางสายเลือดต่อกัน พบว่าไมโครแซท-เทลไลท์ 4 ตัว คือ LaT05, LaT16, LaT17 และ LaT26 มีขนาดระหว่าง 250-500 bp โดยมีจำนวนอัลลีลระหว่าง 4-13 ค่า heterozygosity 0.62-0.88 และค่า Polymorphic Information Content (PIC) 0.56-0.87 พบว่า LaT07 ไม่สามารถเพิ่มจำนวนเมื่อใช้ดีเอ็นเอของช้างไทยได้ ผลการศึกษาสรุปว่า LaT05 และ LaT26 เป็นไมโครแซทเทลไลท์ที่มีประสิทธิภาพสูงในการตรวจหาความสัมพันธ์ทางสายเลือดเนื่องจากมีค่า PIC=0.87 และการใช้ไมโครแซทเทลไลท์จำนวน 4 ตัวที่ศึกษาทำให้การตรวจสอบความสัมพันธ์ทางสายเลือดได้ผลที่ถูกต้องแม่นยำสอดคล้องกับพันธุ์ประวัติถึงร้อยละ 99.74.

คำสำคัญ : ไมโครแซทเทลไลท์ ความหลากหลาย ช้างไทย ความสัมพันธ์ทางสายเลือด

[1]ภาควิชาสัตวบาล, [2]ภาควิชากายวิภาคศาสตร์ คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ถ. อังรีดูนังต์ ปทุมวัน กรุงเทพฯ 10330.
[*]ผู้รับผิดชอบบทความ

Introduction

The Thai elephant belongs to one of three breeds of Asian elephants *(Elephas maximus)*. In Thailand, there are two populations of elephants: those in the wild and those that have been domesticated and reproduced. The dwindling population of Thai elephants has occurred for 40 years and the number currently totals only 5,000 animals (Jintanasonthi, 1999). Based on this, it is highly likely that the number of elephants in Thailand will further plummet unless conservation plans and effort are made. When the population is reduced, probability of inbreeding and the consequential low birth weight, high mortality rate at birth and low fertility at puberty, is very high. Therefore, research and development of techniques required for conservation, especially on genetics of the elephants, are urgently required.

Molecular genetic techniques have been extensively utilized in animal breeding and selection since they provide accuracy and efficiency in individual identification and parentage testing. Determination of polymorphisms of genetic markers can be achieved using tandemly repeated DNA in order to identify the difference in the number of base-pair repetitions (Bruford and Wayne, 1993). Microsatellite loci which belong to Asian elephants *(Elephas maximus)* and African elephants *(Loxodonta africana)* have been investigated (Archie et al., 2003; Comstock et al., 2000, 2002; Eggert et al., 2000; Fernando et al., 2001). However, the number of loci reported is quite limited and, more importantly, those loci were tested in elephants of different regions of the world and they may not represent patterns of microsatellite loci in Thai domestic elephants. Thus, determination and application of microsatellite markers in individual identification and parentage testing will further support the conservation plan of elephants in Thailand.

This investigation has a global objective of studying polymorphisms of microsatellite loci in Thai domestic elephants in order to apply them in identification and parentage control.

Materials and Methods

Source of DNA

Blood samples were collected from 11 elephants of five families with record of genetic relatedness, including one sire, 4 dams and 6 offspring. Additional specimens were collected from 10 genetically unrelated elephants. All elephants were kept and raised in the northern, northeastern and central parts of Thailand.

Source of microsatellite markers

Five polymorphic tetranucleotide microsatellite loci found in African Savannah elephants *(Loxodonta africana africana)* including LaT05, LaT07, LaT16, LaT17 and LaT26 were used (Archie et al. 2003).

DNA analysis

DNA was extracted from whole blood samples using a commercial kit (QIAGEN?). PCR amplification was performed using the primers for 5 loci: LaT05, LaT07, LaT16, LaT17 and LaT26 at 55-56oC for annealing. All PCR products were simultaneously analysed for size using polyacrylamide gel electrophoresis (PAGE). Allele numbers and frequencies were recorded.

Data analysis

Results from each elephant were matched and compared by pair for all microsatellite markers tested. They were consisted of 210 pairs from 21 elephants. Genetic unrelatedness was determined based on absolutely unmatching alleles while the pair that shared 1 or 2 alleles showed genetic relatedness. Allele frequency, heterozygosity, Polymorphic Information Content (PIC) (Botstein et al., 1980), Exclusion Probability (EP) and Combined Exclusion Probability (CEP) were calculated based on the data detected (Ron et al., 1996).

Results

Microsatellite loci amplification by PCR was successful for markers LaT05, LaT16, LaT17 and LaT26 (Fig. 1). However, LaT07 primers could not amplify although PCR conditions were carefully adjusted. Analysis by PAGE revealed marker sizes of 250 ñ 500 bp, and the number of alleles for each microsatellite locus ranged from 4 ñ 13. The allele frequency was between 0.0238 ñ 0.5238 while the heterozygosity and PIC values were 0.6224 ñ 0.8787 and 0.5605 ñ 0.8672, respectively (Table 1).

Results from analysis of alleles for 1-4 markers by matching data from 2 elephants at a time were compared with pedigree and family records. It was found that when 4 markers were analyzed simultaneously, parentage testing results were in accordance with the family history. All 11 elephants were determined to be genetically related in 5 families. In addition, other 10 elephants were shown to be unrelated.

Analysis of CEP values for the accuracy of interpretation is useful when pedigree and family records of the elephants are unclear and ambiguous. In this study, CEP values reflected the efficacy of markers of 99.74%.

Parentage identification using 1-3 markers demonstrated that the probability of genetic relatedness (parent-offspring) could be varied based on the number and type of markers used. It is noteworthy that incorrect interpretations were found in 2 pairs of elephants tested although the accuracy probability was up to 99.41% (based on the calculation of CEP values of using 3 markers). Therefore, using low efficient markers and few number of markers would further the chance of incorrect interpretations (Table 2).

Discussion

The purpose of this investigation was to evaluate the efficiency and accuracy of using microsatellite polymorphisms for parentage control and identification in Thai domestic elephants. Microsatellite loci LaT05, LaT07, LaT16, LaT17 and LaT26 used in this study, were already found to exist in African elephants *(Loxodonta africana africana)* (Archie et al., 2003). We found that LaT05, LaT16, LaT17 and LaT26 (except LaT07), could be

Table 1 Polymorphic data of microsatellite markers

	LaT05		LaT26		LaT16		LaT17	
Allele size (bp)	**350-500**		**350-400**		**250-400**		**250-350**	
No. of alleles	**13**		**11**		**6**		**4**	
Type of Alleles	**n**	**f**	**n**	**f**	**n**	**f**	**n**	**f**
Type A (smallest)	2	0.0476	4	0.0952	18	0.4286	1	0.0238
Type B	6	0.1429	4	0.0952	1	0.0238	9	0.2143
Type C	2	0.0476	4	0.0952	6	0.1429	22	0.5238
Type D	4	0.0952	1	0.0238	5	0.1190	10	0.2381
Type E	1	0.0238	1	0.0238	11	0.2619		
Type F	3	0.0714	2	0.0476	1	0.0238		
Type G	2	0.0476	3	0.0714				
Type H	8	0.1905	7	0.1667				
Type I	3	0.0714	8	0.1905				
Type J	1	0.0238	6	0.1429				
Type K	8	0.1905	2	0.0476				
Type L	1	0.0238						
Type M (largest)	1	0.0238						
heterozygosity	0.8787		0.8776		0.7120		0.6224	
PIC value	0.8672		0.8654		0.6681		0.5605	

n: no. of alleles; f : allele frequency; bp: base pair

Table 2 Comparison of microsatellite marker data in parentage identification

Markers	**No. of markers Used**	**No. of elephants***		**Accuracy****	**Exclusion Efficiency (%)**
		interpreted	**not in accordance with pedigree records**		
LaT16	1	122	112	46.67%	66.81
LaT17	1	107	97	53.81%	56.05
LaT26	1	61	51	75.71%	86.54
LaT05	1	60	50	76.19%	86.72
LaT16-17	2	56	46	78.10%	85.41
LaT05-17	2	41	31	85.24%	94.16
LaT05-16	2	38	28	86.67%	95.59
LaT17-26	2	37	27	87.14%	94.08
LaT16-26	2	33	23	89.05%	95.53
LaT05-16-17	3	26	16	92.38%	98.06
LaT05-26	2	19	9	95.71%	98.21
LaT16-17-26	3	18	8	96.62%	98.04
LaT05-17-26	3	17	7	96.67%	99.21
LaT05-16-26	3	12	2	99.05%	99.41
LaT05-16-17-26	4	10	0	100.00%	99.74

*No. of elephants (pairs) tested using 1-4 microsatellite markers

**Percentage of accuracy in accordance with the pedigree records

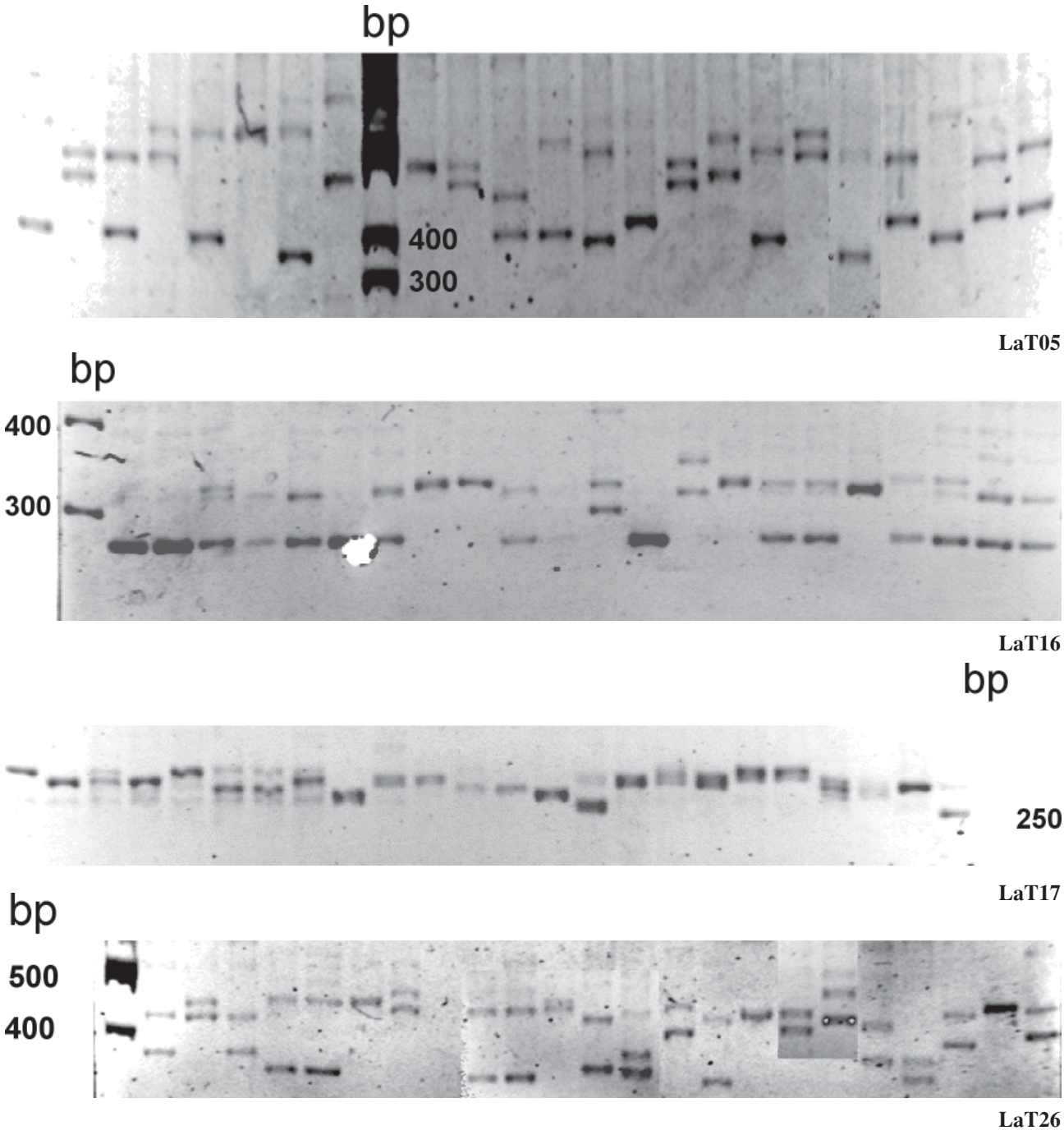

Figure 1 Polymorphic alleles of each microsatellite marker (LaT05, 16, 17 and 26, respectively).

PCR-amplified, suggesting that African and Asian elephants (Elephas maximus) share the conserved regions especially in tandem-repeated DNAs.

Based on a Polymorphic Information Content (PIC) values, LaT05 and LaT26 appear to be the most efficient and desirable markers in parentage identification while LaT16 and LaT17 are of medium efficiency. In addition, Combined Exclusion Probability (CEP) values and Exclusion Efficiency demonstrated that using different markers together could increase the confidence which could be high or low according to markers selected (Baron et al., 2002; Visscher et al. 2002).

Analysis of microsatellite data in parentage identification using 4 markers of LaT05, LaT16, LaT17 and LaT26 was efficient and accurate in that the results of genetic relationship among 11 elephants of 5 families

were in accordance with the family history and pedigree record, with the confidence level of 99.74%. However, the false positive results were found if only 1-3 markers were used though the level of confidence was up to 99.41%. In this study, the microsatellite loci LaT05, LaT26, LaT16 and LaT17 when used together are shown to be efficient and accurate in parentage identification of Thai domestic elephants.

Acknowledgements

This study was financially supported by Faculty of Veterinary Science, Chulalongkorn University 2005 Research Fund and Merial (Thailand) Co. Ltd. We thank the elephant owners in the northern, northeastern and central parts of Thailand who allowed us to collect blood samples.

References

Archie, E.A., Moss, C.J. and Alberts, S.C. 2003. Characterization of tetranucleotide microsatellite loci in the African Savannah elephant *(Loxodonta africana africana)*. Mol. Ecol. Notes. 3: 244-246.

Baron, E.E., Marting, M.L., Verneque, R.S. and Coutinho, L.L. 2002. Parentage testing and effect of misidentification on the estimation of breeding value in Gir cattle. Genet. Mol. Biol. 25: 389-394.

Botstein, D., White, R.L., Skolnick, M. and Davis, R.W. 1980. Construction of a genetic linkage map In man using restriction fragment length polymorphisms. Am. J. Hum. Genet. 32: 182-190.

Bruford, M.W. and Wayne, R.K. 1993. Microsatellites and their application to population genetic studies. Cur. Op. Genet. Devel. 3: 939-943.

Comstock, K.E., Georgiadis, N., Pecon-Slattery, J., Roca, A.L., Ostrander, E.A., OíBrian, S.J. and Wasser, S.K. 2002. Patterns of molecular genetic variation among African elephant populations. Mol. Ecol. 11: 2489-2498.

Comstock, E.K., Wasser, K.S. and Ostrander, A.E. 2000. Polymorphic microsatellite DNA loci identified in the African elephant *(Loxodonta africana)*. Mol. Ecol. 9: 1004-1006.

Eggert, L.S., Ramakrishnan, U., Mundy, N.I. and Woodruff, D.S. 2000. Polymorphic microsatellite DNA markers in the African elephant *(Loxodonta africana)* and their use in the Asian elephant *(Elephas maximus)*. Mol. Ecol. Notes. 9: 2223-2225.

Fernando, P., Vidya, T.N.C. and Melnick, D.J. 2001. Isolation and characterization of tri- and tetra nucleotide microsatellite loci in the Asian elephant *(Elephas maximus)*. Mol. Ecol. Notes. 1: 232-233.

Jintanasonthi, B. 1999. Bringing elephants back to nature. Advanced Thailand Geographic. 4(36): 58-75.

Ron, M., Blanc, Y., Band, M., Ezra, E., and Weller, J.I. 1996. Misidentification rate in the Israeli dairy cattle population and its implications for genetic improvement. J. Dairy Sci. 79: 676-681.

Visscher, P.M., Woolliams, J.A., Smith, D. and Williams, J.L. 2002. Estimation of pedigree erros in the UK dairy population using microsatellite markers and the impact on selection. J. Dairy Sci. 85: 2368-2375.